

**Contest-type competition between age classes in scramble-type *Callosobruchus maculatus* (Coleoptera: Bruchidae)**

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## **Abstract**

We examined the effect of age differences on competition type in individuals of a scramble-type strain of *Callosobruchus maculatus* (F.). When oviposition of two individuals on a bean was manipulated to introduce time intervals using two lines with different adult body colors, the frequency of two-adult emergence decreased with the introduction of sequential oviposition. This result indicates that an age difference between two individuals induces contest competition. The frequency of adult emergence in older individuals decreased, whereas in younger individuals it increased with the introduction of sequential oviposition. Using a dissecting microscope, we observed that bodies of older individuals that died in the bean during the 4-day oviposition interval were crushed at the pupal stage under the pupal chambers of younger individuals. These results show that an age difference between two larvae in a bean causes contest competition due to one-sided interference by a younger individual during pupation of an older individual. Based on these experimental results, we discuss the ecological cause of contest competition and the population-level consequences of identified interactions in scramble-type *C. maculatus*.

**Key words:** bean weevil, competition type, inter-cohort competition, pupation, resource competition.

## INTRODUCTION

Resource competition can be defined in the contest–scramble spectrum according to resource partitioning among competitors (Nicholson 1954). Contest and scramble-type competitions are characterized by resource monopolization and resource sharing, respectively. Contest competition results in a constant number of survivors with enough resource gain against an initial density of competitors, while scramble competition results in an increased number of survivors with decreased resource intake and an increased number of competitors. Outcomes of different patterns of competition have different consequences for population dynamics and the evolution of individual traits (Godfray 1987; Łomnicki 1988; Ives 1989).

Competition types and their outcomes are usually investigated by using larvae of the same age or stage in insects (Prinkkila & Hanski 1995; Jones *et al.* 1996; Guedes *et al.* 2007). However, competition between individuals may take place in insect populations in which individuals of different ages or stages are present concurrently. Individuals of different ages or stages differ in individual traits, such as behavioral, life history, physiological, and morphological traits (Polis 1984; Cornell *et al.* 1987), which can influence the type of competition that results (e.g., Toquenaga & Fujii 1990; Boivin & van Baaren 2000; Alves-Costa & Knogge 2005). Thus, differences in individual traits among competitors of different ages or stages may result in the type of competition among different-aged competitors being different from that among same-aged competitors.

The interaction between individuals of different ages may affect competition type in scramble-type *Callosobruchus maculatus* (F.). Multiple larvae in a bean compete for food resources because the larvae cannot move from one bean to another. Scramble-type larvae in a bean exhibit competition in which they share limited food resources. As multiple

larvae of the same age within a bean are able to access sufficient resources for their survival, several adults emerge (Toquenaga & Fujii 1991; Takano *et al.* 2001).

Differences in the time of oviposition on a bean, however, lead to variations in the age and stage of individuals within the bean. Individuals of scramble-type *C. maculatus* may adopt aggressive behavior against conspecific competitors of different ages or stages. In these cases, variations in the age and stage of individuals may result in interference interactions between individuals of different ages, which may induce contest competition in *C. maculatus* exhibiting scramble-type larval competition.

In this study, we examined the effect of age differences between individuals in a bean on competition type and the underlying mechanism. The age differences may induce contest-type competition. The change in competition type based on patterns of adult emergence from beans with two eggs was examined by increasing the time interval between the first and second ovipositions. We demonstrated a decrease in two-adult emergence from beans with two eggs when the time interval between the first and second ovipositions was increased, due to one-sided interference by a younger individual in the emergence of an older individual. Based on the experimental results, we discuss the ecological cause of the contest-type competition and the population-level consequences of identified interactions in scramble-type *C. maculatus*.

## **MATERIALS AND METHODS**

### **Experimental lines and conditions**

*Callosobruchus maculatus* beetles are stored-product pests found worldwide (Southgate 1979; Jackai & Daoust 1986). They are holometabolous insects; the larvae complete their entire development within beans. Before pupation, a larva makes a pupal chamber

by utilizing the open space created as it feeds on the bean (Larson 1938). Adults emerging from beans initiate mating and ovipositing within a few hours. To distinguish whether an emerging adult represented the first or second larva to enter a bean, we used MS1-5 and the MS1-5 black lines (hereafter called normal and black lines) of the hQ strain of *C. maculatus* in this study. For more detailed information on the normal line, refer to studies by Mano and Toquenaga (2008a, 2008b). The black line was established from recessive black mutants that were newly derived from the normal line.

Females that emerged from *Vigna radiata* (L.) Wilczek beans with one egg were used for oviposition in all experiments. Beans with one hatched egg of each line were obtained by allowing at least 20 females of the line to lay eggs on a layer of *V. radiata* beans in a ventilated glass dish (120 mm in diameter and 30 mm in height) for no more than 2 h followed by removal of the females. Twenty-five days after oviposition, we collected the emerged adults of each line from the beans, which had been kept individually. After mass mating for 24 h in a Petri dish, males were removed and females were used for oviposition. Culturing of stock and all experiments were performed at  $30 \pm 1^\circ\text{C}$ ,  $50 \pm 5\%$  RH, with a photoperiod of 24L:0D. The *V. radiata* beans ( $80.69 \pm 1.53$  mg (mean  $\pm$  SE),  $n = 30$ ) were used as larval resources in all experiments. The beans were large enough for two larvae to complete their development if there was no interaction between them, making it less likely that both larvae would die as a consequence of resource shortage.

### **Life history traits and larval competition types of two lines**

We investigated the relative frequency of adult emergence from *V. radiata* beans with one egg of each line, developmental time, fresh weight of emerged adults, and larval

competition type. More than 20 females were allowed to lay eggs on *V. radiata* beans in a ventilated glass dish for 1 h. Seven days after oviposition, each bean with one or two hatched eggs was placed in a separate cell of a multi-compartment clear plastic box (Nunclon, Roskilde, Denmark), and checked daily for adult emergence. Each newly emerged adult from a bean with one hatched egg was sexed and weighed on an electrobalance. This procedure was repeated five times for each line. In each repetition, we obtained 14–30 beans with one hatched egg each, and 23–24 beans with two hatched eggs each. Based on the patterns of adult emergence from beans with one and two hatched eggs, we estimated a C value to examine larval competition type (Takano *et al.* 2001). The C value is the proportion of larvae that exhibit interference behavior in a bean with two hatched eggs to the number of larvae within a population, ranging from 0 to 1. A C value of 0 indicates that all larvae in a population exhibit scramble-type behavior. In contrast, a C value of 1 indicates that all larvae in a population exhibit contest-type behavior.

### **Competition observed with changes in oviposition time intervals**

To examine whether a delay in oviposition of two individuals on the same bean affects adult emergence, we manipulated oviposition time intervals. More than 20 females of one line were allowed to lay eggs on *V. radiata* beans in a ventilated glass dish for 1 h. All eggs but one were removed from the surface of each bean. The perimeter of each egg was marked using a black marker (Macky, Goku-Boso, Zebra, Inc.) for later identification. After 1 h (hereafter called 0 days), 2 days, or 4 days, more than 20 females of the other line were allowed to lay eggs on the same beans for 1 h. After the second oviposition, surplus eggs were removed to obtain beans with one hatched egg

from each oviposition. Seven days after the second oviposition, each bean with a hatched egg from both ovipositions was kept individually. Reciprocal ovipositing was performed for each time interval (normal → black and black → normal). A total of 24 beans were observed for each treatment except for the case of black females that oviposited 4 days after normal females in which 23 beans were observed. The line of the emerging adults on each bean was recorded based on adult body color. The mechanism underlying the mortality of older individuals due to the interaction between individuals of different ages was examined. Forty days after the second oviposition, each bean from which only the younger individual emerged was dissected under a dissecting microscope to find the dead body of the older individual and check its developmental stage.

### **Statistics**

All data were analyzed with R version 2.11.2 (R Development Core Team 2010). Because there was no significant heterogeneity among replicates, the results of five replicates were pooled to analyze the frequency of adult emergence from the beans, the proportion of males as an index of sex ratio, developmental time, and fresh weights of adults emerging from the beans. Bayesian interval estimations of the frequency of adult emergence from *V. radiata* beans with one egg of each line and the sex ratio were determined for comparison between normal and black lines. Bayesian ANOVA was used to examine the effects of line, sex, and their interaction with adult fresh weight, developmental time, and C value (Qian & Shen 2007; Qian 2010). Detailed methods for Bayesian ANOVA have been included in the supplemental material (see Appendix S1 in Supporting Information).

To examine changes in patterns of adult emergence from beans with two hatched eggs with varying oviposition time intervals for a given oviposition pattern, the probabilities of adult emergence patterns for different oviposition time intervals for a given oviposition pattern were inferred. The adult emergence patterns can be classified into one of four possible classes: no adults, older individual, younger individual, and two adults. Therefore, we used a multinomial distribution for our multinomial data, in which the chance of an adult emergence pattern being classified into a given class is a specified probability (McCarthy 2007). This procedure was performed using data on patterns of adult emergence from beans from each of the oviposition time intervals (0, 2, and 4 days) for each oviposition pattern (normal → black and black → normal).

For all Bayesian inferences, the computation was implemented using a Markov chain Monte Carlo simulation. Calculations were performed using the MCMCpack package for the frequency of adult emergence from beans with one egg of each line, the sex ratio, and patterns of adult emergence from beans with two hatched eggs (Martin *et al.* 2010). Bayesian ANOVA was conducted using lmer and mcmcSamp of lme4 package (Bates & Maechler 2010; Qian 2010).

## **RESULTS**

### **Life history traits and larval competition types of two lines**

The estimated frequencies of adult emergence from *V. radiata* beans with one egg were 0.89: 0.83 to 0.94 (mean: 95% credible interval) and 0.90: 0.84 to 0.95 in normal and black lines, respectively ( $n = 120$  for the normal line,  $n = 117$  for the black line). Results indicate that the frequency of adult emergence did not differ significantly between the two lines. Estimated male proportions were 0.45: 0.36 to 0.55 and 0.57: 0.47 to 0.66 in

normal and black lines, respectively ( $n = 49$  for normal female,  $n = 59$  for normal male,  $n = 60$  for black female, and  $n = 46$  for black male). Results indicate that sex ratios of normal and black lines did not deviate from 1:1. Table 1 shows adult fresh weight, developmental time, and the estimated C value for each sex of normal and black lines. Results of Bayesian ANOVA showed no significant effects of line, sex, or their interaction on adult fresh weight, developmental time, and C value, except that males weighed slightly less than females in both lines. Detailed results of Bayesian ANOVA have been included in the supplemental material (see Appendices S1-S4 in Supporting Information).

### **Competition observed with changes in oviposition time intervals**

Figure 1 summarizes the estimated probabilities for patterns of adult emergence from beans with two hatched eggs for different oviposition time intervals for the two oviposition patterns of normal  $\rightarrow$  black and black  $\rightarrow$  normal. If the credible interval of the probability for a pattern of adult emergence does not overlap among oviposition intervals, the frequency of the adult-emergence pattern differs among the oviposition intervals. For the oviposition pattern of normal  $\rightarrow$  black, the frequency of two-adult emergence was smaller for the 4-day oviposition interval than for the 0-day oviposition interval (Fig. 1 A). Similarly, the frequency of two-adult emergence was smaller for the 4-day oviposition interval than for the 0-day and 2-day oviposition intervals for the oviposition pattern of black  $\rightarrow$  normal. For the oviposition pattern, the frequency of two-adult emergence did not differ between 0-day and 2-day oviposition intervals. On the other hand, for each oviposition pattern, the frequency of the first oviposited individual failing to emerge from the bean from which the second oviposited individual

emerged was higher for the 4-day oviposition interval than for the 0-day and 2-day oviposition intervals (Fig. 1 C). For each oviposition pattern, the frequency did not differ between 0-day and 2-day oviposition intervals. The other frequencies for each oviposition pattern did not differ among oviposition intervals (Fig. 1 B and D).

The numbers of first oviposited individuals that died in beans from which the second oviposited individuals emerged for the 4-day oviposition interval were 13 and 12 for the normal and black lines, respectively. Of the 13 and 12 dead individuals for the normal and black lines, 12 and 11 individuals, respectively, were pupae.

Examination of the states of older individuals that died in the beans at the pupal stage for the 4-day oviposition interval revealed that all of them had their body parts crushed by the pupal chambers of younger individuals (Fig. 2). These results indicate that the first oviposited individual might be killed during the pupal stage by the second oviposited larva constructing a pupal chamber at the 4-day oviposition interval.

## **DISCUSSION**

This study demonstrates that a difference in age causes one-sided interference by a younger individual of larva in the emergence of an older individual of pupa, resulting in contest competition at the 4-day oviposition interval. Our results indicate that interaction between individuals of different ages induces contest-type competition type in *C. maculatus* exhibiting scramble-type larval competition. The ecological cause of the contest competition and the population-level consequences of identified interactions in scramble-type *C. maculatus* are discussed below.

Food is considered to be the main limiting resource that causes competition among immature *C. maculatus* individuals, when multiple larvae are present in a bean

(Toquenaga & Fujii 1991; Guedes *et al.* 2007). In the present study, because the younger individual killed the older individual in the pupal stage, and, since *C. maculatus* larvae are not cannibalistic, the younger individuals used only the remaining food resources in beans irrespective of whether older individuals were alive or dead. Therefore, food resources may not be the cause of contest competition by one-sided interference at the 4-day oviposition interval.

One hypothesis has been proposed for the ecological cause of contest competition due to one-sided interference by a younger individual in emergence: contest competition for space may be induced by a long oviposition time interval. When two eggs are oviposited simultaneously, the two hatching individuals may have a greater chance of interacting with each other during the larval stage (Mano & Toquenaga 2008a, 2008b). The larvae of the normal line avoid interference by making a wall-like structure (Mano & Toquenaga 2008b), thereby partitioning the space within the bean. Dividing the space in this way allows both larvae to emerge from the same bean. On the other hand, during the 4-day oviposition interval, the first oviposited individual makes a pupal chamber using the open space created by feeding on the bean without encountering the second individual. Figure 2 shows a dead first oviposited individual at the center of a bean. Larvae of normal and black lines feed at the center of a bean when only a single larva is present. The first oviposited larva may make a pupal chamber across the center of the bean, leaving only the periphery available. This prevents a later oviposited individual from making a pupal chamber in a *V. radiata* bean. Even if the second oviposited larva eats around the periphery, the open space created may be too small for the larva to make a pupal chamber without encroaching on the first individual. Therefore, the second oviposited individual might kill the first one at the pupal stage by

crushing it with its pupal chamber. Further experiments are required in order to test this hypothesis.

Messina (1991) demonstrated a decrease in the number of emerged adults with longer oviposition time intervals in the IITA strain exhibiting scramble-type larval competition. Two hypotheses have been proposed for the mechanism underlying this phenomenon. The first is that the bean size is so small that younger individuals may die of starvation with too small a peripheral part available to them. The second hypothesis is that an increase in the mortality of the first oviposited individual caused by the second one may result in a decrease in the number of adults emerging from a bean with long oviposition time intervals in the IITA strain of *C. maculatus*, as shown by the present study. Scramble-type geographic strains of *C. maculatus* are found worldwide (Takano *et al.* 2001). Other strains exhibiting scramble-type larval competition may also show an increase in the mortality of older individuals at the pupal stage in a bean.

*Callosobruchus maculatus* has been widely used as a model organism in population dynamics studies (Fujii 1968; Ishii & Shimada 2008). Studying the patterns of adult emergence against the initial number of larvae helps in understanding the mechanism underlying the population dynamics of *C. maculatus* (Fujii 1968; Toquenaga *et al.* 1994). To predict population dynamics, previous studies have examined the pattern of the number of emerged adults against the initial number of *C. maculatus* individuals using larvae of the same age (Guedes *et al.* 2007; Toquenaga & Fujii 1991). The effect of interaction among individuals of different ages on the pattern of adult-emergence outcomes against the initial number of competitors has been largely ignored. Our study, however, suggests that the interaction between individuals of different ages may be an important factor decreasing the success of adult emergence in

*C. maculatus* exhibiting scramble-type competition. Our findings also suggest that when larvae of different ages share a bean, older larvae may fail to emerge because earlier pupation makes them vulnerable to attack by younger larvae. This may lead to a delay in the increase in the number of adults and, in turn, to an increase in generation time.

On the other hand, in order to examine the reproduction curve of *C. maculatus* for estimating the population dynamics, the pattern of the number of emerged adults against the initial adult number of *C. maculatus* has been examined by allowing females to lay eggs on beans until they died (e.g., Fujii 1968; Ishii & Shimada 2008). Our results suggest that if the experimental manipulation of female oviposition for long time induces the interaction between individuals at different ages in beans, the pattern of the reproduction curve may be affected by the interaction. The pattern of the reproduction curve in the presence of the interaction between individuals at different ages may differ from that in the absence of the interaction. The relationship between the pattern of reproduction curve of *Callosobruchus* species and the larval competition type were sometimes discussed (e.g., Smith & Lessells 1985; Toquenaga & Fujii 1990). The relationship may be complicated by whether or not individuals at different ages interact with each other in beans. Therefore, the interaction between individuals of *C. maculatus* at different ages in a bean should be given more attention.

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**Table 1 Adult fresh weight, developmental time, and C value of each sex of normal and black lines (mean  $\pm$  SD) with number of samples (in parentheses)**

	Normal		Black	
	Female	Male	Female	Male
Adult Fresh	6.26 $\pm$ 0.54	4.85 $\pm$ 0.44	5.95 $\pm$ 0.55	4.38 $\pm$ 0.35
weight (mg)	(n = 49)	(n = 59)	(n = 60)	(n = 46)
Developmental	21.61 $\pm$ 1.00	21.49 $\pm$ 1.39	22.85 $\pm$ 1.66	22.52 $\pm$ 1.07
time (days)	(n = 49)	(n = 59)	(n = 60)	(n = 46)
C value	0.13 $\pm$ 0.10	0.08 $\pm$ 0.07	0.04 $\pm$ 0.03	0.04 $\pm$ 0.03
	(n = 5)	(n = 5)	(n = 5)	(n = 5)

## Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1: Detailed methods and results for Bayesian ANOVA

Appendix S2: Bayesian information criterion values for the models of adult fresh weight, developmental time, and C value

Appendix S3: Results of Bayesian ANOVA for adult fresh weight

Appendix S4: Results of Bayesian ANOVA for developmental time

Appendix S5: Results of Bayesian ANOVA for the C value

## Figure legends

**Figure 1** Estimated probabilities of beans with (A) two-adult, (B) older individual, (C) younger individual, and (D) no adult emergences. Open and filled circles indicate means for normal line oviposition before black line oviposition, and black line oviposition on beans before normal line oviposition, respectively. Bars represent 90% credible intervals. For each oviposition pattern in (A) and (C), credible intervals with different lowercase letters above the bars did not overlap each other.

**Figure 2** Photograph of an older individual that died due to being crushed under the pupal chamber of a younger individual at a 4-day oviposition interval.

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Figure 1

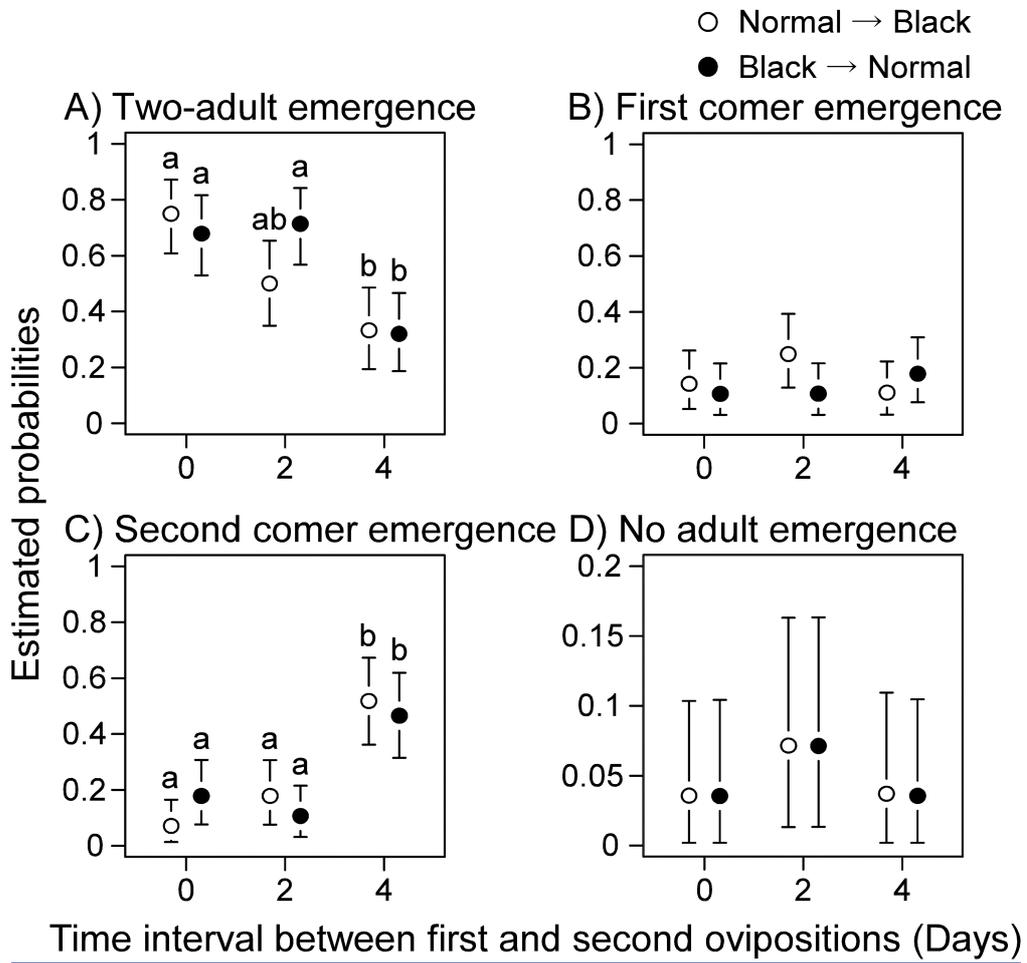
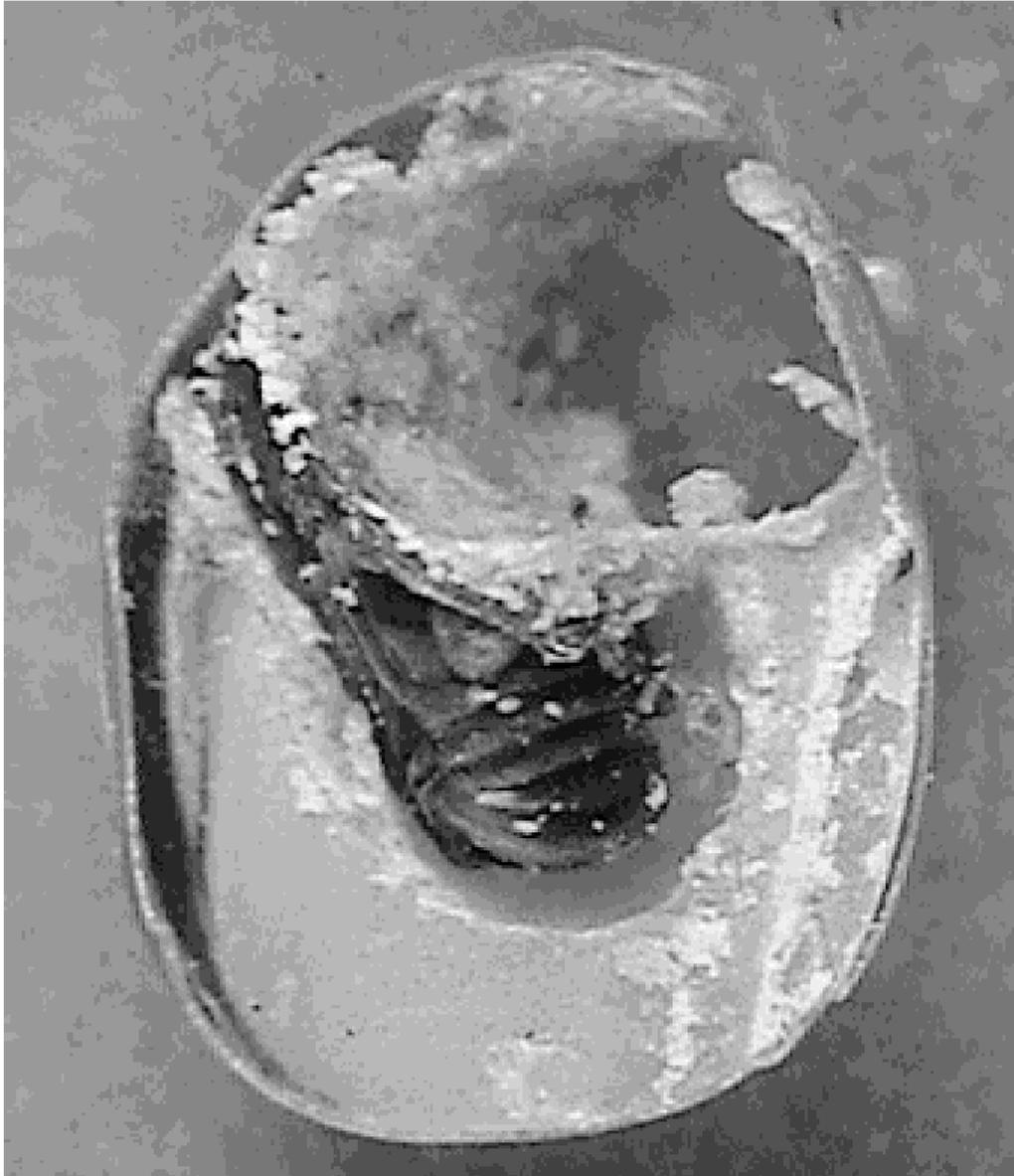


Figure 2



1 mm



# Contest-type Competition Between Age Classes in Scramble-type *Callosobruchus maculatus* (Coleoptera: Bruchidae)

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## Supporting Information

### Appendix S1 Detailed methods and results for Bayesian ANOVA

Bayesian ANOVA was used to examine the effects of line, sex, and their interaction with developmental time, fresh weight, and C value. Bayesian ANOVA can be summarized as the estimation of variance components and treatment effects using hierarchical regression (Qian & Shen 2007; Qian 2010).

Total response variable variance is partitioned into components representing treatment and other factors. Full models of Bayesian ANOVA for developmental time, fresh weight, and C value can be expressed as

$$Y_{ijk} = \beta_0 + \beta_{1i} + \beta_{2j} + \beta_{3ij} + \varepsilon_{ijk}$$

where  $Y$  is developmental time, adult fresh weight, or C value;  $\beta_0$  is the overall mean;  $\beta_{1i}$  is the effect of line  $i$  (normal or black);  $\beta_{2j}$  is the effect of sex  $j$  (female or male); and  $\beta_{3ij}$  is the effect of the interaction between line  $i$  and sex  $j$ .  $\varepsilon_{ijk}$  is the residual term, where  $k$  is the index of individual observations within each line–sex treatment cell. Each of the coefficients  $\beta_{1i}$ ,  $\beta_{2j}$ ,  $\beta_{3ij}$ , and  $\varepsilon_{ijk}$  is assumed to have a normal distribution with mean 0 and a constant variance. Total variances in developmental time, fresh weight, and C value were partitioned into the following four components: line, sex, their interaction, and residuals. The inferences on the coefficient values and variances were drawn using Bayesian posterior distributions of the parameters. We compared four models (model 1:  $\beta_0 + \beta_{1i} + \beta_{2j} + \beta_{3ij} + \varepsilon_{ijk}$ ; model 2:  $\beta_0 + \beta_{1i} + \beta_{2j} + \varepsilon_{ijk}$ ; model 3:  $\beta_0 + \beta_{1i} + \varepsilon_{ijk}$ ; and model 4:  $\beta_0 + \beta_{2j} + \varepsilon_{ijk}$ ) for each of the parameters (i.e., developmental time, fresh weight, and

C value). The Bayesian Information Criterion (BIC) was used as a model selection criterion. The model with the smallest BIC value for each of the parameters was selected as the most parsimonious model. A smaller BIC value for a particular model relative to BIC values for alternative models indicates that the model provides a better description of the data than alternative models.

Of the four models for adult fresh weight, model 2, which included strain and sex effects, had the smallest BIC value (Appendix S2). The variance component of the line term was smaller than that of the residual term (Appendix S3A). Estimated posterior 95% credible intervals for the effect of normal and black lines on adult weight did not deviate from zero (0.19: -1.00 to 1.39 (mean: 95% credible interval) for the normal line, and -0.19: -1.38 to 1.01 for the black line; see Appendix S3B). The effect of line was not detected in adult fresh weight. The variance component of the sex term was the largest of the three variance components (Appendix S3A). Estimated 95% credible intervals for the effects of female and male (sex) deviated slightly from zero (0.73: 0.00 to 1.49 for females, and -0.73: -1.48 to 0.01 for males, in Appendix S3C). This indicates that males weighed slightly less than females in both lines.

The model for developmental time with the smallest BIC value was model 3, which showed the effect of line (Appendix S2). The variance component of the line term was smaller than that of the residual term (Appendix S4A). Estimated posterior 95% credible intervals for the effect of normal and black lines for adult weight did not deviate from zero (-0.57: -3.97 to 2.76 for the normal line, and 0.57: -2.79 to 3.93 for the black line, in Appendix S4B). The effect of line was not detected in developmental time.

For C value, model 3, which showed the effect of line, had the smallest BIC

value (Appendix S2). The variance component of the line term did not differ from that of the residual term (Appendix S5A). Estimated posterior 95% credible intervals for the effect of normal and black lines for adult weight did not deviate from zero (0.02: -0.14 to 0.20 for the normal line, and -0.02: -0.20 to 0.14 for the black line, in Appendix S5B). Results indicate that no effect of line was detected in the C value.

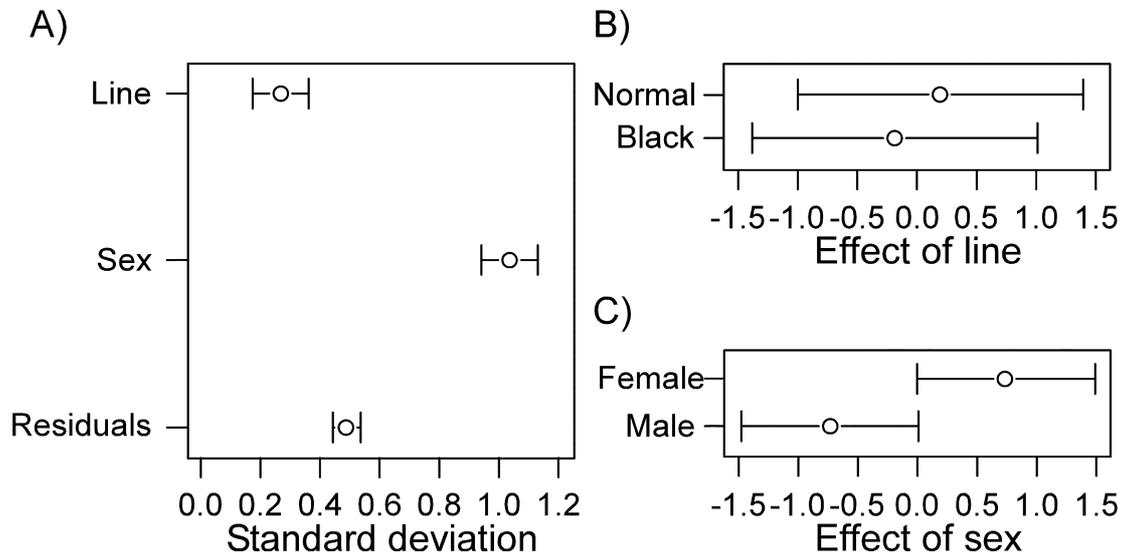
**Appendix S2 Bayesian information criterion values for the models of adult fresh weight, developmental time, and C value**

Label	Model	Adult fresh weight	Developmental time	C value
1	$L^a + S^b + L \times S$	333.7	762.7	-31.03
2	$L + S$	328.5	757.3	-34.02
3	L	573.6	752.1	-37.02
4	S	351.1	783.1	-34.99

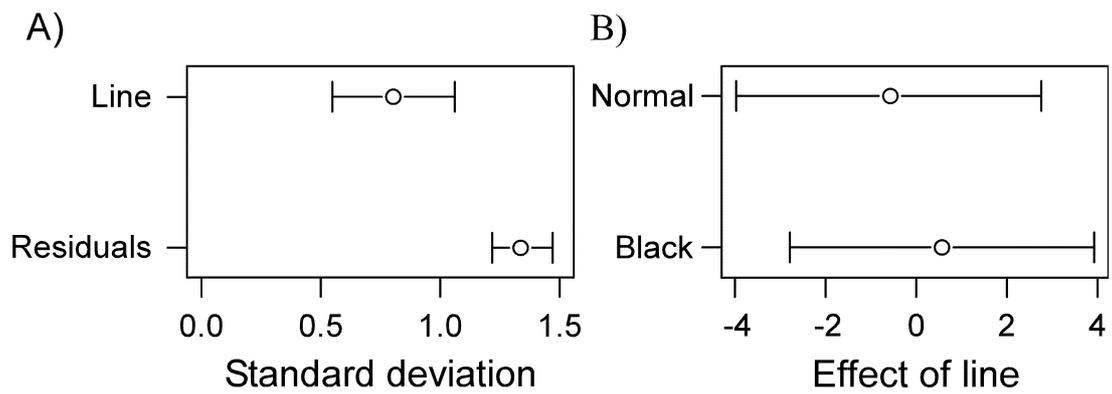
<sup>a</sup>L indicates the effect of line.

<sup>b</sup>S indicates the effect of sex.

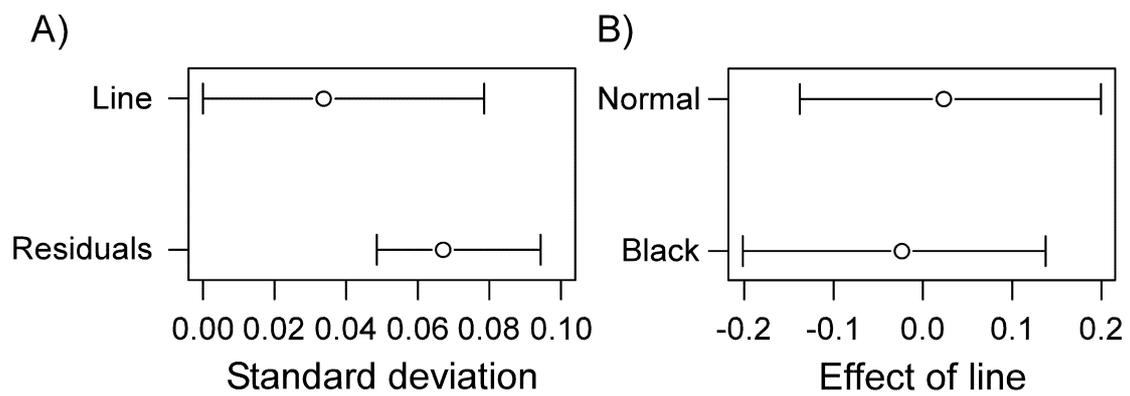
**Appendix S3** Results of Bayesian ANOVA for adult fresh weight. (A) Estimated standard deviation of estimated variance components. (B) Estimated effects of line on adult fresh weight. (C) Estimated effects of sex. Circles are estimated posterior means; bars represent the 95% credible intervals.



**Appendix S4** Results of Bayesian ANOVA for developmental time. (A) Estimated standard deviation of estimated variance components. (B) Estimated effects of line on developmental time. Circles are estimated posterior means; bars represent the 95% credible intervals.



**Appendix S5** Results of Bayesian ANOVA for the C value. (A) Estimated standard deviation of estimated variance components. (B) Estimated effects of line on the C value. Circles are estimated posterior means; bars represent the 95% credible intervals.



## REFERENCES

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Qian SS, Shen Z (2007) Ecological applications of multilevel analysis of variance. *Ecology* **88**, 2489–2495.